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Research article

CRF_1 but not glucocorticoid receptor antagonists reduce separation-induced distress vocalizations in guinea pig pups and CRF overexpressing mouse pups. A combination study with paroxetine☆,☆☆

P. Monika Verdouw [a,1], Joantine C.J. van Esterik [a,1], Bernard W.M.M. Peeters [b], Mark J. Millan [c], Lucianne Groenink [a,*]

[a] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Brain Center Rudolf Magnus (BCRM), P.O. Box 80082, 3508 TB Utrecht, The Netherlands
[b] Department of Anatomy, Radboud University Medical Center Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
[c] Institut de Recherches Servier, Croissy-sur-Seine, France

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ABSTRACT

Rationale: Given the large number of patients that does not respond sufficiently to currently available treatment for anxiety disorders, there is a need for improved treatment.
Objectives: We evaluated the anxiolytic effects of corticotropin releasing factor $(CRF)_1$ receptor antagonists and glucocorticoid receptor (GR) antagonists in the separation-induced vocalization test in guinea pigs and transgenic mice with central CRF overexpression. Furthermore, we explored effects of these drugs when given in combination with a suboptimal dose of a selective serotonin re-uptake inhibitor (SSRI).
Methods: In guinea pig pups, the CRF_1 receptor antagonists CP-154,526 and DMP695, and the GR antagonists mifepristone and Org34517 (all at 2.5, 10 and 40 mg/kg intraperitoneally (IP)) were tested alone or in combination with 0.63 mg/kg paroxetine IP. In CRF overexpressing mouse pups and wild type littermates, effects of CP-154,526 (10, 20 and 40 mg/kg subcutaneously (SC)) and mifepristone (5, 15, 45 mg/kg SC) were studied alone or in combination with 0.03 mg/kg paroxetine SC.
Results: CRF_1 but not GR antagonists reduced the number of calls relative to vehicle in guinea pigs and mice, independent of genotype. Treatment of CRF_1 receptor or GR antagonists with paroxetine had no combined effect in guinea pigs, wild type or CRF overexpressing mice.
Conclusions: Current results indicate robust anxiolytic properties of CRF_1 receptor antagonists in guinea pigs and mice overexpressing CRF, and lack thereof of GR antagonists. Although no combined treatment effects were observed, it would be interesting to study combined treatment of CRF_1 receptor antagonists with SSRIs following chronic drug administration.

1. Introduction

Serotonin re-uptake inhibitors (SSRIs) are among the most frequently described anxiolytics (Baldwin et al., 2014). However, problems encountered with the existing anxiolytics include the large number of non-responders to drug treatment, the delayed onset of action and adverse effects (Stewart et al., 2015). Therefore, there is a need for more effective pharmacotherapy. Such improved pharmacological treatment may be achieved by multi-target drug strategies (Hendriksen and Groenink, 2015; Millan, 2006).

Anxiety disorders are complex disorders as many factors are involved in their pathogenesis. Persistent changes in corticotropin-releasing factor (CRF), a neuropeptide with central and peripheral effects on anxiety, may contribute to the development of anxiety disorders

Abbrevations: CRF, corticotropin-releasing factor; CRFtg, CRF transgenic; DEC-ABC, Animal Ethical Committee of the Academic Biomedical Centre Utrecht; GR, glucocorticoid receptor; HPA axis, hypothalamus-pituitary-adrenal axis; PND, postnatal day; SSRI, serotonin re-uptake inhibitor; USV, ultrasonic vocalization.

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* Corresponding author.
E-mail address: l.groenink@uu.nl (L. Groenink).

[1] J.C.J. van Esterik and P.M. Verdouw contributed equally to this work.

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(Heim and Nemeroff, 1999) and is known to play a role in anxiety-like behavior in both humans and rodents (Bale, 2005). CRF_1 receptors are found throughout the brain, including the cerebral cortex, extended amygdala, septum, and brainstem, but in high density in the pituitary (Schmidt et al., 2010; Van Pett et al., 2000). CRF_1 receptors in the pituitary regulate the hypothalamus-pituitary-adrenal (HPA) axis. Activation of the HPA axis by CRF triggers glucocorticoid secretion (e.g. cortisol, corticosterone). In the central nervous system, corticosterone exerts its effect among others through lower-affinity glucocorticoid receptors (GRs). GRs are widely expressed in the hypothalamus and areas that regulate emotions including prefrontal cortex, basolateral amygdala, and hippocampus (Alt et al., 2010; Joëls and de Kloet, 1994).

Considering the role of CRF in the regulation of anxiety, anxiolytic activity of CRF_1 receptor antagonists has been studied and effects are observed depending on test, species and compound under study (Kehne and Cain, 2010). For instance, the non-peptidergic CRF_1 receptor antagonists CP-154,526 and DMP695 both revealed anxiolytic effects in a lick suppression and social interaction test, but not in an elevated plus maze and conditioned vocalization test in adult rats (Millan et al., 2001). Anxiolytic effects of GR antagonists have been less well studied, and reported effects also depend on the specific test, species and compound under study (Korte et al., 1996; Loi et al., 2015; Pugh et al., 1997). In this respect, the GR antagonist mifepristone showed anxiolytic effects in the rat elevated plus maze test immediately after the exposure to a conditioned stressor, but not in the fear-motivated immobility test (Korte et al., 1995). Another potentially interesting GR antagonist is Org34517. Org34517 is a weaker but more selective GR antagonist than mifepristone. In contrast to mifepristone, Org34517, only has limited affinity towards progesterone receptors (Peeters et al., 2008; Sitruk-Ware and Spitz, 2003) and behaves as a full GR antagonist without partial agonist activity (Havel et al., 1996; Peeters et al., 2008). Although to our knowledge Org34517 has not been studied in anxiety tests, Org34517 and mifepristone both attenuated anxiety associated with ethanol withdrawal in rats (Reynolds et al., 2015; Sharrett-Field et al., 2013). A pilot study with 60^+ humans diagnosed with anxiety disorder observed improvements in worry severity, memory and executive function in individuals with higher baseline levels of cortisol after 3–4 weeks of treatment with mifepristone (Lenze et al., 2014). To date, early clinical trial results of both CRF_1 receptor and GR antagonists have been disappointing (Schatzberg, 2015; Stewart et al., 2015), but more extensive studies are required to determine potentially beneficial effects in anxiety patients with HPA axis dysregulation.

Interestingly, several lines of research have shown interactions between CRF and serotonin in the regulation of anxiety-like responses (Lukkes et al., 2009; Meloni et al., 2008). As such, combined treatment of (low dose) SSRIs with CRF_1 receptor antagonists could be beneficial in the treatment of anxiety disorders. For example, CP-154,526 abolished fear acquisition deficits in serotonin transporter knockout rats (Bijlsma et al., 2015). In addition, a study of Heitland et al. (2016) showed an interaction between genetic variants of the CRF_1 receptor and serotonin transporter with regard to human fear acquisition deficits. Combined administration of SSRIs with GR antagonists may offer another potentially beneficial approach for the treatment of anxiety disorders. For instance, corticosterone may modulate emotional behavior indirectly by altering serotonergic neurotransmission (Judge et al., 2004; Linthorst and Reul, 2008). Microdialysis studies in rats support the potentially beneficial effects of combined treatment of SSRIs with GR antagonists. Combined chronic treatment with the GR antagonist Org34850 enhanced the ability of the SSRI fluoxetine to elevate forebrain serotonin levels (Johnson et al., 2007). A subsequent study showed that this effect could be attributed to downregulation of serotonin transporters (Johnson et al., 2009). However, the behavioral effect of CRF_1 receptor antagonists or GR antagonists in combination with SSRIs has not yet been tested in animal models for anxiety.

Here we used the separation-induced distress vocalization test in guinea pigs and mice to determine anxiolytic effects of the treatment conditions of interest. Separation-induced distress vocalization occurs in a wide variety of species, including man. It is an innate emotional response exhibited after a short period of separation from parents, in particular the mother (Pettijohn, 1979). A recent meta-analysis showed that in guinea pigs the test detects a wide range of drug classes in use for the treatment of anxiety disorders, including SSRIs and other antidepressants, making it a valuable screen to study compounds with anxiolytic potential (Groenink et al., 2015).

In light of the above, we determined the effects of the selective CRF_1 receptor antagonists, CP-154,526 and DMP695, and the GR antagonists, mifepristone (i.e. RU486, RU38486) and Org34517, on distress vocalizations in guinea pig pups and in transgenic mouse pups overexpressing central CRF (CRFtg; Dirks et al., 2002a). These transgenic mice have elevated central CRF levels, elevated basal plasma corticosterone levels and a dysregulated HPA axis (Groenink et al., 2002), reminiscent of changes observed in certain human anxiety disorders (Arborelius et al., 1999), and were used to model anxiety disorder with HPA axis dysregulation. In addition, we explored the effects of combined administration of these antagonists with a suboptimal dose of the SSRI paroxetine. We chose to test a suboptimal dose of paroxetine since we expected synergistic effects of this multi-target treatment. Also, in a clinical setting the advantage of a multi-target strategy would be to achieve treatment efficacy by combining lower doses of drugs, which would meanwhile reduce incidence of side effects and circumvent initial anxiogenic effects of SSRIs (Hendriksen and Groenink, 2015; Millan, 2006).

2. Materials and methods

2.1. Animals, breeding and housing conditions

2.1.1. Guinea pigs

Female guinea pigs (*Cavia porcellus*, HsdPoc:DH) were obtained six weeks pregnant (Harlan Laboratories, Venlo, The Netherlands). Eight weeks after arrival, pups were weaned and female dams were re-used for in-house breeding with male guinea pigs (HsdPoc:DH, Harlan Laboratories, Venlo, The Netherlands). All experiments were performed with pups from in-house breeding, except for the Org34517 experiments which were performed with pups from pregnant dams at arrival. Both male and female pups were used for the vocalization experiments.

All pregnant females were maintained on a 12 h/12 h light/dark cycle (lights on from 7:00 AM–7:00 PM), an ambient temperature of 21 ± 1 °C and relative humidity (40–60%). Female guinea pigs were housed in pairs in standard guinea pig cages ($725 \times 620 \times 300$ mm, base surface 4200 cm^2) provided with hay, a shelter and food (Guinea Pig FDI, SDS Diets, UK) and bottled tap water ad libitum. Female pairs remained in these cages throughout gestation and lactation. Each female gave birth to 1–7 (typically 3–5) pups. Occasionally, pups did not survive birth, likely because their body weight was either too low (underdevelopment/too many pups) or too high (asphyxiation during slow birth). A total of 24 breeding pairs and 151 pups were used for the vocalization experiments. Body weight of guinea pig pups, the day before their first vocalization test, ranged from 101 g–264 g. The Animal Ethical Committee of the Academic Biomedical Centre Utrecht (DEC-ABC) approved of all experiments prior to the onset of the study under permit number 2007.I.11.28.

2.1.2. CRFtg mice

Transgenic mice overexpressing neural CRF were generated as described previously (Dirks et al., 2002b). Briefly, the CRF transgene was composed of the complete coding sequence of rat CRF cDNA (0.6-kb fragment), which was inserted into a 8.2-kb genomic DNA-fragment encompassing the murine Thy-1.2 gene, including regulatory regions and polyadenylation signal sequence. The Thy-1 regulatory sequences drive constitutive transgene expression in postnatal and adult neurons. Subsequent breeding at the local breeding facilities (Utrecht, The Netherlands) consisted of mating between heterozygous transgenic males